

COMPONENT PART NOTICE

THIS PAPER IS A COMPONENT PART OF THE FOLLOWING COMPILATION REPORT:

TITLE: Peptides. Chemistry and Biology: Proceedings of the American Peptide Symposium
(12th) Held in Cambridge, Massachusetts on 16-21 June 1991.

TO ORDER THE COMPLETE COMPILATION REPORT, USE AD-A256 113.

THE COMPONENT PART IS PROVIDED HERE TO ALLOW USERS ACCESS TO INDIVIDUALLY AUTHORED SECTIONS OF PROCEEDING, ANNALS, SYMPOSIA, ETC. HOWEVER, THE COMPONENT SHOULD BE CONSIDERED WITHIN THE CONTEXT OF THE OVERALL COMPILATION REPORT AND NOT AS A STAND-ALONE TECHNICAL REPORT.

THE FOLLOWING COMPONENT PART NUMBERS COMPRISE THE COMPILATION REPORT:

AD#: AD-P008 306 AD#: AD-P008 592
AD#: _____ AD#: _____
AD#: _____ AD#: _____

Accession For	
NTIS <input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
DTIC TAB <input type="checkbox"/>	<input type="checkbox"/>
Unannounced <input type="checkbox"/>	<input type="checkbox"/>
Justification	
By _____	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	

Novel trifluoromethyl-containing peptides as inhibitors for angiotensin-converting enzyme and enkephalin-aminopeptidase

Iwao Ojima^a, Fabian Jameison^a, Koji Kato^a, John D. Conway^a, Bela Peté^a,
Alexandra Graham-Ode^a, Kazuaki Nakahashi^b, Masaki Hagiwara^b,
Hans E. Radunz^c and Christine Schittenhelm^c

^aDepartment of Chemistry, State University of New York at Stony Brook,
Stony Brook, NY 11794-3400, U.S.A.

^bFuji Chemical Ind. Ltd., 530 Chokeiji, Takaoka, Toyama 933, Japan

^cE. Merck AG, Pharmazeutische Chemie, Frankfurter Straße 250, 4119,
D-6100 Darmstadt 1, Germany

Introduction

There is an increasing interest in the incorporation of fluorinated amino acids as well as fluoro-isosteres into physiologically active peptides and enzyme inhibitors [1]. We have been interested in the unique lipophilicity, size and polarity of trifluoromethyl (TFM) group. In the present study, we examined the effects of TFM on the activity and specificity of angiotensin-converting enzyme (ACE) inhibitors and enkephalin analogs.

Although many analogs of the potent ACE inhibitors, captopril and enalaprilat, have been synthesized, there is a paucity of information in the literature regarding the synthesis and activity of fluorinated congeners [2]. Accordingly, it would

Table 1 ACE inhibitory activity of trifluoromethyl-containing peptides

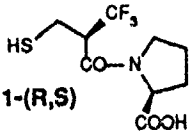
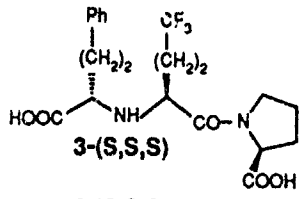
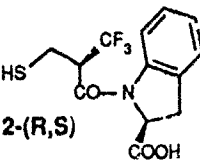
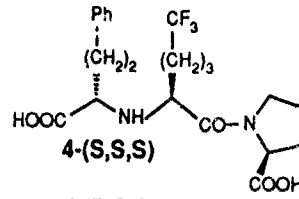
Inhibitor	IC ₅₀ (M)	Inhibitor	IC ₅₀ (M)
 1-(R,S)	3 x 10 ⁻¹⁰	 3-(S,S,S)	6 x 10 ⁻⁸
1-(S,S)	5 x 10 ⁻⁷	3-(R,S,S)	1 x 10 ⁻³
 2-(R,S)	8 x 10 ⁻⁸	 4-(S,S,S)	3 x 10 ⁻⁸
2-(S,S)	6 x 10 ⁻⁶	4-(R,S,S)	2 x 10 ⁻⁴

Table 2 Analgesic activity of TFM-enkephalins (*i.c.v.*, mouse)

Enkephalins	ED ₅₀ (mol/mouse)	Enkephalins	ED ₅₀ (mol/mouse)
Tyr-D-TFNV-Gly-Phe-Met-NH ₂	7.5 × 10 ⁻¹²	Tyr-Gly-L-TFNV-Phe-Met-NH ₂	2.2 × 10 ⁻⁸
Tyr-D-Nval-Gly-Phe-Met-NH ₂	3.5 × 10 ⁻¹¹	Tyr-L-TFNV-Gly-Phe-Met-NH ₂	2.5 × 10 ⁻⁸
Tyr-Gly-L-TFNV-Phe-Met-NH ₂	1.4 × 10 ⁻⁷	Methionine-Enkephalin	7.0 × 10 ⁻⁷
Tyr-Gly-D-TFNV-Phe-Met-NH ₂	1.2 × 10 ⁻⁸	Tyr-D-Ala-Gly-Phe-Met-NH ₂	4.5 × 10 ⁻¹¹
Tyr-D-TFNL-Gly-Phe-Met-NH ₂	6.5 × 10 ⁻¹¹	Morphine · HCl	7.0 × 10 ⁻¹¹
Tyr-D-TFNV-Gly-(N-Me)Phe-Met-NH ₂	2.0 × 10 ⁻¹²	Sedapain TM	5.0 × 10 ⁻¹¹

be of interest to examine the influence that TFM incorporation might have on such ACE inhibitors.

Enkephalins and their analogs have been extensively studied regarding their analgesic activity as well as functions as neurotransmitter [3]. However, no systematic study has been performed on the incorporation of TFM-amino acids into enkephalins. Thus, we looked at the effects of TFM-amino acids on analgesic activity (*in vivo*) as well as receptor binding ability (*in vitro*) so that we can distinguish the effects based on receptor binding from inhibition of degradation by endogenous enzymes.

Results and Discussion

A series of new TFM-containing peptides are synthesized as potential inhibitors for ACE, which are TFM analogs and homologs of captopril and enalaprilat (Table 1). As Table 1 shows, the direct substitution of TFM for methyl provides a very potent captopril analog, 1-(R,S). The SAR study by means of the π -SCF-molecular mechanics program (PIMM) developed by Lindner [4] as well as the MM calculations of active site conformations by SYBIL 5.0 program indicates that 1-(R,S) should be at least 5 times better than (S,S)-captopril (IC₅₀ = 4 × 10⁻⁹M), and the latter calculation suggests that 1-(R,S) should be more than 1000 times better than 1-(S,S), which are consistent with the observed results. Stereoelectronic effects on conformation and lipophilicity of TFM would account for the excellent activity of 1-(R,S). Incorporation of both TFM and an indoline residue unexpectedly gives a less potent captopril analog, 2-(R,S). Enalaprilat analogs derived from replacement of the alanine residue with (S)-TFM-norvaline (L-TFNV) [5], 3-(S,S,S), and (S)-TFM-norleucine (L-TFNL) [5] residues, 4-(S,S,S), gave moderately potent peptides. The other diastereomers of 2-4 exhibited 2-5 order of magnitude weaker activities as predicted.

A series of TFM-containing enkephalin analogs were synthesized and their *in vivo* analgesic activity determined (Table 2). These modified enkephalins are derived from replacement of (a) Gly² and Gly³ by D-TFNV, L-TFNV and D-TFNL and (b) Phe⁴ by (N-Me)Phe⁴. As Table 2 shows, [D-TFNV², Met-NH₂⁵]- and [D-TFNV², (N-Me)Phe⁴, Met-NH₂⁵]-enkephalins prove to be extremely potent *in vivo* with respect to methionine-enkephalin (Met-Enk) (10⁵-fold stronger). The *in vitro* binding assay to μ -, δ -, and κ -receptors revealed that [D-TFNV²,

Table 3 Receptor binding assay for [D-TFNV², Met-NH₂⁵]enkephalin (D-TFNV-Met-Enk)

Enkephalins	Receptor	Tissue	Ligand ^a	IC ₅₀ (M)
D-TFNV-Met-Enk	μ	cerebrum (rat)	[³ H]-PL-017	5 × 10 ⁻¹⁰
Met-Enk	μ	cerebrum (rat)	[³ H]-PL-017	2 × 10 ⁻⁹
D-TFNV-Met-Enk	δ	cerebrum (rat)	[³ H]-DPDPE	2 × 10 ⁻⁹
Met-Enk	δ	cerebrum (rat)	[³ H]-DPDPE	1 × 10 ⁻⁹
D-TFNV-Met-Enk	k	cerebellum (guinea pig)	[³ H]-U-69593	4 × 10 ⁻⁷
Met-Enk	k	cerebellum (guinea pig)	[³ H]-U-69593	> 1 × 10 ⁻⁵

^a [³H]-PL-017 = [³H]Tyr-Pro-MePhe-D-Pro-NH₂; [³H]-DPDPE = [³H][D-Pen², D-Pen⁵]enkephalin; [³H]-U-69593 = [³H] (5α,7α,8β)-(-)-N-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5)non-8-yl]benzene acetamide.

Met-NH₂⁵]enkephalin is only several times better binder to μ-site than Met-Enk (Table 3). Therefore, it is concluded that the observed remarkable increase in potency is mainly due to the inhibition of degradation by enkephaline-aminopeptidase.

Acknowledgements

This work was supported by grants from Center for Biotechnology sponsored by New York State Science and Technology Foundation, National Institute of Health (NIGMS), and Ajinomoto Co., Inc. Generous support from Japan Halon Co., Ltd. is also gratefully acknowledged.

References

1. Welch, J.T., *Tetrahedron*, **43** (1987) 3123.
2. Thorsett and Wyvratt, M.J., In Turner, A.J. (Ed.) *Neuropeptides and Their Peptidases*, VCH, New York, 1987, p. 229.
3. Hansen, P.E. and Morgan, B.A., In Udenfried, S. and Meienhofer, J. (Eds.) *Peptides*, Vol. 6, Academic Press, New York, 1984, p. 269.
4. Lindner, H.J., *Tetrahedron*, **30** (1974) 1127.
5. Ojima, I., Kato, K., Nakahashi, K., Fuchikami, T. and Fujita, M., *J. Org. Chem.*, **54** (1989) 4511.